Attorney Docket No. 9013-67 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Foster et al.
Application No.: 10/518,471
Int'l Filing Date: December 17, 2004
For: *REMOVAL OF PRION INFECTIVITY*

Confirmation No.: 7924
Group Art Unit: 1746
Examiner: Bibi S. Carrillo

Declaration of Ian MacGregor, Ph.D. Pursuant to 37 C.F.R. § 1.132

I, Ian R. Mac Gregor, do hereby declare and say as follows:

1. I have a Ph.D. in Biochemistry, from University of London (King's College Medical School). I am Lead Scientist (Consultant Level Research Scientist), in the Products & Components Research Group for the Scottish National Blood Transfusion Service, Edinburgh. I am involved in prion research and have authored or co-authored more than 16 publications related to this area. A *curriculum vitae* is attached herewith at Appendix B.

2. I have read and am familiar with the contents of the James et al. publication (U.S. Application Serial No. 2003/0162225) cited by the Examiner in connection with the '471 application.

3. James et al. discusses treatment of recombinant prion proteins and not abnormal, infectious proteins, which are the subject of the present invention. It is well known that there are significant structural differences between normal and abnormal prions. In fact, it is these structural differences, which give rise to the abnormal infectious behaviour of infectious prion proteins. Thus, behaviour which could be ascribed to normal prion proteins could not be regarded as a good basis for treatment of abnormal prion proteins. These differences are discussed below in greater detail.

4. Differences in properties are attributed to differences in conformation since sequences and secondary structures are the same.

It is generally accepted that the primary and secondary structures of normal and infectious prion proteins are the same. However their physico-chemical properties are quite different and this is attributed to their differing tertiary structures. While both forms have a

disordered N terminus, the globular C domain of an infectious prion protein has much more β-pleated sheet structure than that of a normal prion. Conversely, normal prion protein has much more α-helix (Turk *et al.*, 1889; James *et al.*, 1997). These differences are reflected in different exposure of hydrophobic regions of the molecule and in turn in different physico-chemical properties. In addition, normal prion protein is soluble in non-ionic detergent while, in contrast, infectious prion protein is insoluble. Normal prion protein is readily digested by the broad specificity proteinase K while only the N-terminus of infectious prion protein is degraded with the major part of the molecule remaining intact. Further evidence for differences in conformation is given by the inability of a monoclonal antibody, 3F4, directed to an epitope expressed in normal prion protein, to bind to infectious prion protein. However, upon denaturation of the latter, binding to 3F4 is obtained (Safar *et al.*, 1998).

Furthermore, sodium phosphotungstic acid has been used to selectively precipitate infectious prion protein in the presence of normal prion protein. A 10% NaCl solution also will allow enrichment of infectious prion protein from brain homogenate (Polymenidou *et al.*, 2002).

5. A 'most infectious particle' has been characterized and its properties can be distinguished from normal prion protein in several ways.

Infectious prion protein is often found in fibrils. However, recently it has been shown that the most infectious prion protein particles are not fibrillar but are of 17-27 nm (300-600 kDa) while prion infectivity and prion converting activity was virtually absent in oligomers of ≤ 5 prion molecules (Silveira *et al.*, 2005). In contrast, normal prion protein exists in nature mainly as a monomer although some dimeric forms have also been reported (Meyer *et al.*, 2000).

6. Procedures have been developed by which the normal prion protein can be converted to forms similar to infectious forms, underlining the differences.

Protein misfolding cyclic amplifications has been used to convert substrate normal hamster prion protein into infectious prion protein. The substrate prion was not infectious but the generated prion protein, which shared the properties of infectious protein regarding proteinase K resistance, was infectious when injected into hamsters. (Castilla *et al.*, 2005).

7. A number of ways of discriminating normal from infectious prion have been devised. These illustrate the differences in the properties of the two forms.

Antibodies which have specificity for infectious prion protein over normal prion protein have been described. These utilize differences in the conformation of the two forms or the fact that the former is multimeric. Peptoids and chemical ligands have been used in a similar way, using conditions that allow binding of infectious but not normal prion protein.

8. Reference List

All references listed are attached at Appendix C.

Castilla, J., Saa, Ph., Hetz, C., & Soto, C. (2005) In vitro generation of infectious scrapie prions. *Cell*, **121**, 195-206.

James, T.L., Liu, H., Ulyanov, N.B., FarrJones, S., Zhang, H., Donne, D.G., Kaneko, K., Groth, D., Mehlhorn, I., Prusiner, S.B., and Cohen, F.E., Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. *Proceedings of the National Academy of Sciences of the United States of America*, **94**(19), 10086-10091, 1997.

Meyer, R.K., Lustig, A., Oesch, B., Fatzer, R., Zurbriggen, A., and Vandevelde, M. A monomer-dimer equilibrium of a cellular prion protein (PrPC) not observed with recombinant PrP. *J. Biol. Chem.*, **275**(48), 38081-38087, 2000.

Polymenidou, M., Verghse-Nikolai, S., Groshup, M., Chaplin, M.J., Stack, M.G., Plaitakis, A. & Sklaviadis, T. (2002) A short purification process for quantitative isolation of PrP^{SC} from naturally occurring and experimental transmissible spongiform encephalopathies. *BMC.Infect.Dis.*, **2**, 1-8.

Safar, J., Wille, H., Itri, V., Groth, D., Serban, H., Torchia, M., Cohen, F.E. & Prusiner, S.B. (1998) Eight prion strains have PrPSc molecules with different conformations. *Nature Medicine*, **4**, 1157-1165.

Silveira, J.R., Raymond, G.J., Hughson, A.G., Race, R.E., Sim, V.L., Hayes, S.F., & Caughey, B. (2005) The most infectious prion protein particles. *Nature*, **437**, 257-261.

Turk, E., Teplow, D.B., Hood, L.E., & Prusiner, S.B. (1988) Purification properties of the cellular and scrapie hamster prion proteins. *European Journal of Biochemistry*, **176**, 21-30.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Ian MacGregor

Ian R. MacGregor, Ph.D.

5th December 2006

Date